USER friendlyTM construction of vectors for targeted gene replacement in fungi

Frandsen, Rasmus John Normand; Andersson, Jens A.; Giese, Henriette

Published in:
Abstract Book of Plant Biotech Denmark Annual Meeting 2008

Publication date:
2008

Document version
Publisher's PDF, also known as Version of record

Plant Biotech Denmark 2008
POSTER ABSTRACT

USER friendly™ construction of vectors for targeted gene replacement in fungi

Rasmus J.N. Frandsen, Jens A. Andersson and Henriette Giese
Section for Genetics and Microbiology
Institute for Ecology
Faculty of Life Sciences
University of Copenhagen
Denmark

Functional genetics in filamentous fungi has always been dependent on the isolation or construction of mutant strains. The genome sequencing of several different filamentous fungi has changed the experimental approach from forward to reverse genetics. This has speeded up research in areas such as secondary metabolism and pathogenicity factors, thereby increasing the need for faster methods to construct targeted replacement and overexpression mutants. To accommodate this we have developed a new vector system that allows single-step construction of vectors for targeted gene replacement, thereby cutting vector construction time from ten to only three days and removing half of the required work load.
The vector system is dependent on the Uracil-Specific Excision Reagent cloning technology (USER friendly™), which in its commercial version offers high efficient directional cloning of a single PCR amplicon. However, our research shows that USER friendly™ cloning also can be used for the simultaneous directional cloning of several PCR amplicons and vector fragments, with a cloning efficiency of 85 %, thus allowing single-step construction of replacement vectors.
The new vector system includes vectors for: gene replacement (pRF-HU2), promoter exchange (pRF-HU2E), ectopic overexpression (pRF-HUE) and general purpose cloning (pRF-HU). All compatible with both protoplast and *Agrobacterium tumefaciens* mediated transformation technologies.